



Article

A New Species of *Monstrilopsis* Sars, 1921 (Copepoda: Monstrilloida) with an Unusually Reduced Urosome

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Abstract: Male monstrilloid copepods, described herein as *Monstrilopsis paradoxa* sp. nov., were collected from the Chuja Islands, Jeju, Korea, using a light trap. They display many of the common features of *Monstrilopsis*, including large, prominent eyes, an anteriorly positioned oral papilla, and four setae on each caudal ramus. Type-2 modification of the antennules further supports the assignment of the new species to *Monstrilopsis*. However, the present specimens have an unusually low number of urosomal somites, just three in total, compared to five in males of all congeneric species, and from four (in *Cymbasoma*) to five in males of all other monstrilloid genera. Up until now, in the Monstrilloida only females of *Cymbasoma* have been known to have as few as three urosomal somites.

Keywords: Monstrillidae; *Monstrilopsis paradoxa* sp. nov.; *Monstrilopsis planifrons*; morphological taxonomy; tagmosis; male genitalia; pore pattern; male/female matching; marine invertebrate host; semi-parasitic; Korea

1. Introduction

Monstrilloida Sars, 1901 is a small group of marine copepods that comprise approximately 170 nominal species in seven genera currently considered as valid: *Monstrilla* Dana, 1849; *Cymbasoma* Thompson, 1888; *Monstrilopsis* Sars, 1921; *Maemonstrilla* Grygier & Ohtsuka, 2008; *Australomonstrilopsis* Suárez-Morales & McKinnon, 2014; *Caromiobenella* Jeon, Lee & Soh, 2018; *Spinomonstrilla* Suárez-Morales, 2019 [1–7]. This group is distinguished from other copepods by their peculiar life cycle and strange morphology. They have a protelean life-cycle consisting of an endoparasitic juvenile phase and a planktonic adult stage. The juveniles are known to infect various marine invertebrates such as polychaetes, gastropods and bivalve mollusks [8–18]. The adults, often regarded as an exclusively reproductive stage, lack all mouth parts as well as the antennae and are non-feeding.

Most of the recent work on monstrilloids has focused on taxonomy and the morphological description of adults, but studies on the parasitic life stages and host-parasite interactions have been on the increase [2,17–19]. As well, research into monstrilloid diversity has been expanding into lesser-studied regions. For example, Suárez-Morales and McKinnon [6,20] recently described 34 species of four genera from Australian waters, with a promised treatment of similar numbers of Australian *Monstrilla* still to come, and such findings imply the possible occurrence of large numbers of undescribed taxa in other such areas as well.

East Asia is clearly a region where the monstilloid fauna has not been yet fully revealed [16,21]. Serious research on monstilloids in Korea began less than a decade ago with the first record of *Cymbasoma striifrons* Chang, 2012. So far nine species in four genera have been reported from Korea's eastern and southern coastal waters [3,22–26]. The site of the present study, the Chuja Islands, is a group of 42 small islands, four of which are inhabited. They are located between Jeju Island and the mainland of Korea and are affected by the Tsushima Warm Current. Temperate and subtropical creatures coexist there [27,28], leading to the expectation of high biodiversity in their surrounding waters. Here we report a new type of monstilloid with a novel and unusual morphological feature for the group, and described it as a new species.

2. Materials and Methods

A plankton sample was collected by Min Ho Seo (Marine Ecology Research Center, Korea) using a hand-made polyvinyl chloride (PVC) light trap containing an light emitting diode (LED) flashlight as a light source. The type materials of the present new species were collected from 19:00 to 7:00 on 11–12 September 2017 alongside a wharf at the type locality (Figure 1). The captured contents were filtered using a 63 μm mesh test sieve, and the retained material was immediately washed with 99.5% ethanol. The sample was initially fixed with 99.5% ethanol on-site and the fixative was exchanged for fresh 99.5% ethanol in the laboratory. Monstilloids were sorted out from the bulk collection under a SMZ645 stereomicroscope (Nikon, Tokyo, Japan) and kept refrigerated at 4 °C. The osmotic shock of ethanol fixation caused the cephalothorax of many specimens to collapse, so monstilloid specimens used for morphological analysis were exposed to 0.25% trisodium phosphate dodecahydrate solution ($\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$; Daejung Chemicals and Metals, Siheung, Korea) to restore its original shape [3,12,31]. An Eclipse 80i compound microscope with a drawing tube and differential interference optics was used for preparing illustrations. The holotype specimen was dissected into five parts, and each was mounted on a slide separately with lactophenol for detailed examination. Body measurements were obtained by using AxioVision LE64 software (AxioVs40x64 v 4.9.1.0; Carl Zeiss, Oberkochen, Germany) based on the illustrations of the type material. Terminology from Grygier and Ohtsuka [2] and Jeon, et al. [32] was used to describe body segmentation and antennular setation patterns, respectively.

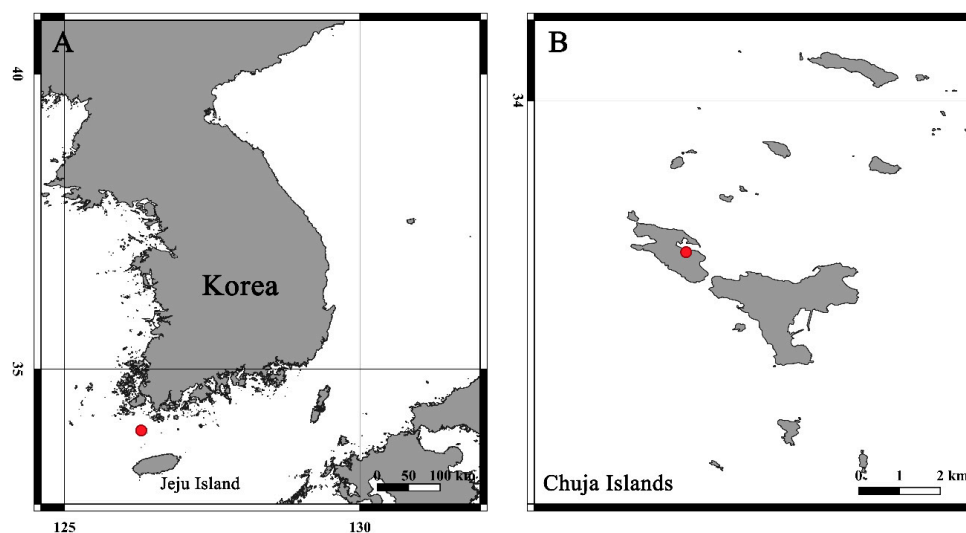


Figure 1. Maps showing the investigated area and the sampling locality. (A) Location of Chuja Islands (red dot), Korea; (B) sampling site (red dot) in Chuja Islands. The maps were prepared using QGIS [29], a free and open-source geographic information system, with the OpenStreetMap data [30].

3. Results

3.1. Systematics

Order Monstrilloida Sars, 1901

Family Monstrillidae Dana, 1849

Genus *Monstrillopsis* Sars, 1921

Monstrillopsis paradoxa sp. nov.

Figures 2–4

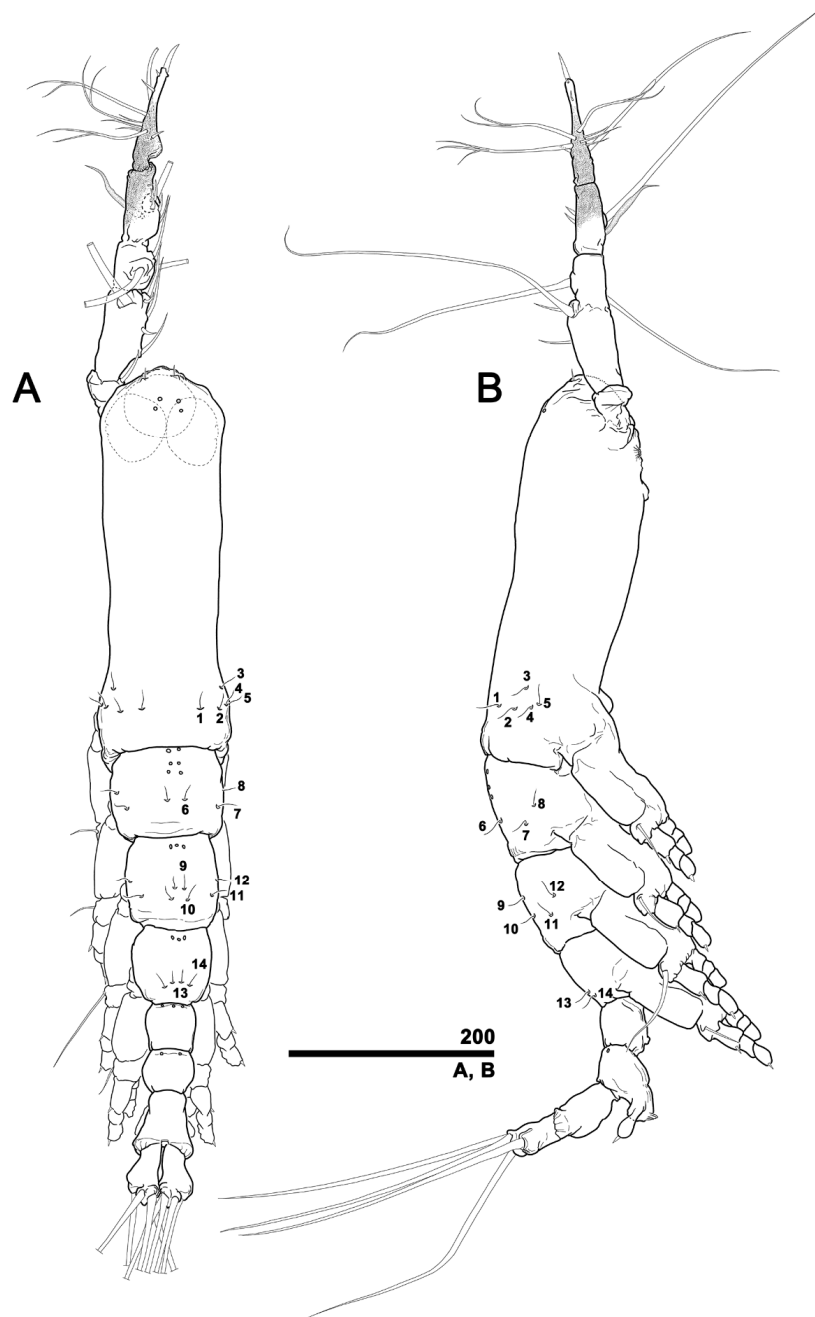


Figure 2. *Monstrillopsis paradoxa* sp. nov., male holotype (MABIK CR00246526) (A) Habitus with pit-setae 1–14 of right side indicated, dorsal; (B) habitus, lateral. Scale bars are in micrometers.

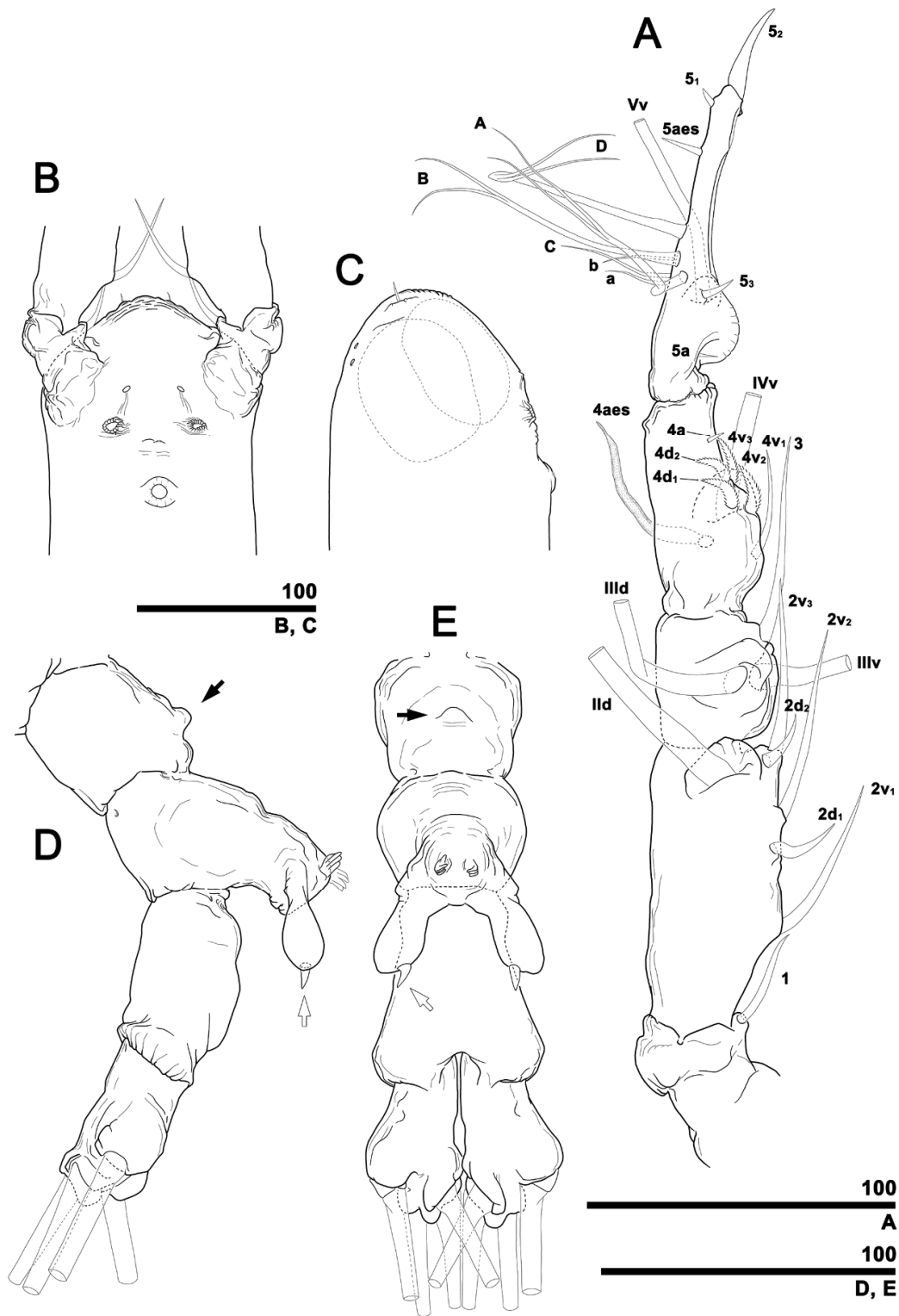


Figure 3. *Monstrillopsis paradoxa* sp. nov., male holotype (MABIK CR00246526). (A) Antennule, left, dorsal; (B) anterior ventral part of cephalothorax showing crumpled margin; (C) anterior part of cephalothorax, lateral; (D) urosome showing ventral protuberance (black arrow) on first urosomal somite and apical spinous element (white arrow) on genital lappet, lateral; (E) urosome showing ventral protuberance (black arrow) on the first urosomal somite and apical spinous element (white arrow) on right genital lappet. Scale bars are in micrometers.

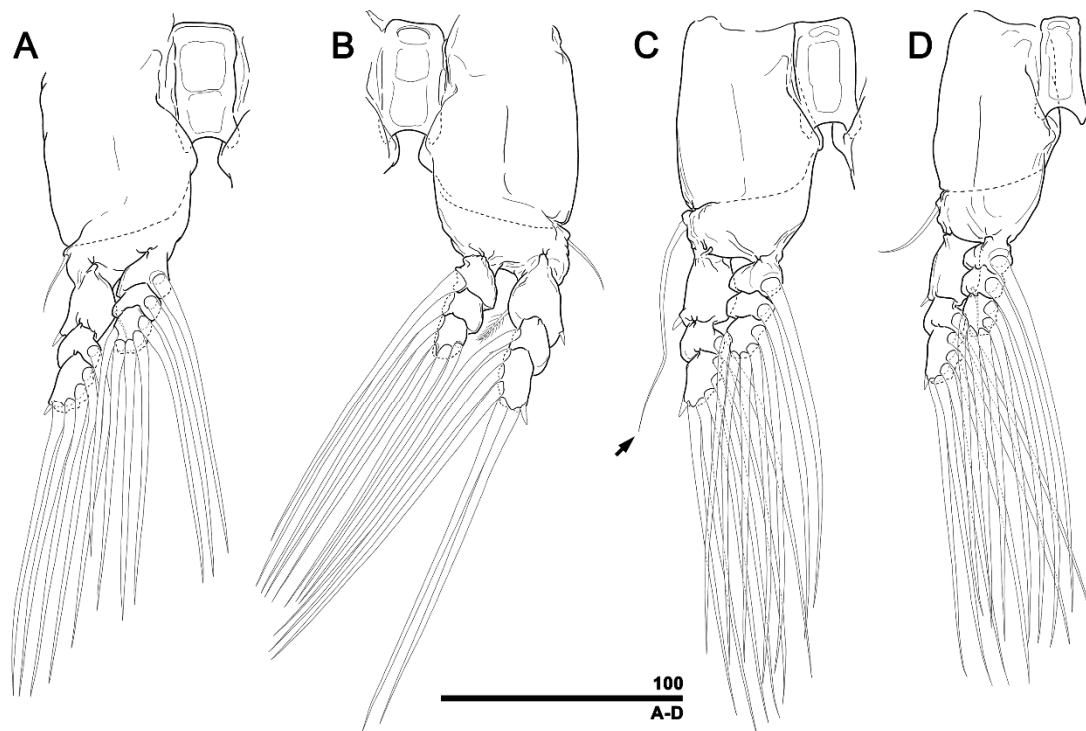


Figure 4. *Monstrillopsis paradoxa* sp. nov., male holotype (MABIK CR00246526). (A) Leg 1 with intercoxal sclerite, right, anterior; (B) leg 2 with intercoxal sclerite, left, anterior; (C) leg 3 showing long basal seta (arrow) and intercoxal sclerite, right, anterior; (D) leg 4 with intercoxal sclerite, left, anterior. Scale bars are in micrometers.

3.2. Type Locality

Yeongheung-ri (33°57.59" N, 126°17.82" E), Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea. English equivalents of political divisions in Korea: ri = village; myeon = township; si = city; do = province.

3.3. Type Material Examined

The male holotype (MABIK CR00246526; dissected and body parts mounted on five separate slides) and five intact paratypes (MABIK CR00246527; in a 99.5% ethanol vial) are deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seochon, Korea.

3.4. Species Diagnosis. Male

Anterior end of cephalothorax rugose with transverse striations. Antennule 5-segmented with geniculation between fourth and fifth segments. Fifth segment showing type-2 modification [13]: inner proximal one-third with hyaline bump, rest of distal part thin, elongate. Two distal segments dark brown in ethanol-preserved specimens (coloration becoming fainter with time, and immediate partial bleaching occurring upon exposure to Na_3PO_4 solution and/or lactophenol): outer lateral side of the fourth segment most strongly pigmented; the fifth segment with gradual distal weakening of pigmentation, completely lacking pigment near the tip. Outermost natatory seta on the third exopodal segment of all legs smooth, lacking typical serration along its outer side. Outer basal seta on third legs extremely long, well exceeding its exopod. Anterior dorsum of first free pediger with three pairs of pores arrayed in two rows flanking midline. Succeeding three somites, i.e. second and third free pedigers and first urosomal somite, each with a transverse row of three pores near the anterior dorsal margin, with middle pore on midline. Urosome consisting of three somites, viz., limbless fourth

free pediger (= first urosomal somite) and genital somite followed directly by anal somite, with no post-genital or penultimate (= preanal) somites. First urosomal somite lacking fifth legs, but with small posteroventral protuberance instead. Genital somite with ventral genital apparatus consisting of a shaft and two lappets. Shaft robust with abrupt notch at midlength in lateral view, more distal part slightly thinner than proximal part and bearing two hand-shaped opercular flaps anteriorly at tip. Genital lappets diverging from posterior distal part of the shaft, clavate in lateral view, rather lamelliform in ventral view. Each lappet armed with a short, robust spinous element at the inner distal corner. Caudal rami twice as long as wide, each with four setae. All caudal setae subequal in length, with inner ventral seta almost always extending diagonally downwards. Measurements: total body length ranged from 0.66–0.88 mm (mean 0.78 mm; $N = 6$ for all measurements reported here). Length ratio (means, with ranges in parentheses) of cephalothorax, metasome, and urosome 49.0 (46.6–53.2):34.1 (30.2–36.6):16.9 (15.9–19.6) in lateral view. Oral papilla located 25.9% (24.3–27.4%) back along the ventral side of cephalothorax. Length of antennules in relation to total body length 45.1% (42.1–51.0%), and to length of cephalothorax 91.9% (86.0–97.0%). Ratio of antennular segment length from proximal to distal 12.3 (11.8–13.8):23.8 (21.7–26.6):14.2 (11.9–16.3):19.9 (19.4–20.4):29.8 (28.9–30.4).

3.5. Description of Male Holotype (MABIK CR00246526)

Total body length 0.76 mm in dorsal view, 0.78 mm in lateral view (Figure 2A,B). Body consisting of cephalothorax incorporating first pediger, free somites 1–3, first urosomal somite, genital somite, and anal somite. Length of somites as percent of total body length 49:12:12:9:6:5:7 in dorsal view, 49:14:12:9:5:4:7 in lateral view. Cephalothorax cylindrical, 0.37 mm long in dorsal view, 0.38 mm long in lateral view, accounting for almost half of total body length. Anterior margin convex, its surface corrugated with strong transversal striations. Anterior part of the forehead with two short, thin sensilla. Width of cephalothorax almost unvarying along its length: narrowest (= waist width; 0.11 mm) at 76.5%, widest (= incorporated first pediger; 0.13 mm) at 91.9% of distance from anterior end. Anterior one-fourth nearly fully occupied by one ventral and two lateral eyes (Figure 2A, Figure 3C). Ventral eye located more anteriorly than lateral eyes, with 1.29 times greater diameter than the latter (70.3 μm versus 54.5 μm). Dorsal surface at the level of eyes with at least four pores in the trapezoidal array (Figure 2A). Ventral surface at the same level with pair of pores and pair of scars (Figure 3B); pores located medially between antennular bases, and scars slightly behind of antennular bases but still medial to them. Moderately developed oral papilla located behind scars, 26% back from anterior margin of cephalothorax. Tergite of incorporated first pediger with five pairs of pit-setae *sensu* Grygier and Ohtsuka [19]: one pair (no. 1) situated dorsally, four pairs laterally (nos. 2–5).

Body somites from first pedigerous somite to genital somite with several pores in various places (Figure 2A,B). First free pediger with three pairs of pit-setae posteriorly (nos. 6–8: one pair dorsally, two pairs laterally) and two longitudinal rows of four pores each, arranged in pairs across midline, with most anterior pore pair covered by posterior cuticular extension of cephalothorax. Second free pediger with four pairs of pit-setae posteriorly (nos. 9–12: two pairs dorsally, two pairs laterally) and three simple pores aligned transversally across anterior midline. Third free pediger with two pairs of pit-setae posteriorly (nos. 13, 14), aligned transversely across dorsum, and transverse row of three simple pores on anterior dorsum. The first urosomal somite also with three simple pores on anterior dorsum and with protuberance near the posterior end of the ventral side. Genital somite with a pair of simple pores in anterior dorsum.

Antennules 5-segmented, with geniculation between fourth and fifth segments (Figure 3A). Length 0.35 mm excluding apical spine 5₂, equal to 44.1% of total body length, 90.2% of cephalothorax length. Length ratio of segments 12:26:12:20:30. First antennular segment with spinous element 1 on inner distal corner, arising slightly dorsally. Second segment armed with five spinous elements (2v_{1–3}, 2d_{1,2}) plus long, strap-like, biplumose seta II_d. Ventral spinous elements (2v-series) well developed, slightly longer than dorsal ones (2d-series). Third segment with three elements (3, III_d, III_v); spinous element 3 located distally on inner side, long III_v and III_d setae extending ventrally and

dorsally, respectively, from midlength of segment. Fourth segment with eight elements ($4v_{1-3}$, $4d_{1,2}$, $4a$, IVv , $4aes$): naked spinous element $4v_1$ thin, twice as long as other $4d$ -, $4v$ -elements; four spinous elements $4v_{2,3}$ and $4d_{1,2}$ all robust, pinnate, curved toward bearing segment; spinous element $4a$ minute, arising at inner distal one-third of segment; IVv and $4aes$ arising from ventral surface of segment, $4aes$ more proximally. Fifth antennular segment modified: inner proximal margin with semicircular expansion, rest of distal part relatively thin, elongate, and twice as long as proximal expansion (Figure 3A). Inner side of distal elongated part with thin, film-like hyaline edge. Segment armed with 12 setal elements. Among these, spinous element 5_1 short, located on outer distal margin; apical spinous element 5_2 elongate, 26% as long as bearing segment; inner spinous element 5_3 arising from dorsal distal part of inner expansion; minute spinous element $5a$ arising from proximal part of expansion; and long, strap-like seta Vv arising from ventral surface at level of spinous element 5_3 . Six outer setae present at midlength of fifth segment: four branched setae (A–D) and two short, simple setae (a, b). Branched seta C rather simple, significantly shorter than other branched setae but still longer than two simple setae. Aesthetasc ($5aes$) located on the outer side of the segment's distal quarter, longer than spine 5_1 . Fourth antennular segment dark brown, especially prominent along the outer lateral side. Proximal half of fifth segment except for hyaline expansion also dark, but pigmentation fading distally, completely absent at tip.

Genital somite rounded in dorsal view, with robust genital shaft constricted at midlength so as to appear notched in lateral view (Figure 2A, Figure 3D,E). Distal part armed with two hand-shaped opercular flaps. Pair of genital lappets diverging from posterior distal part of the shaft, clavate in lateral view, relatively flat and wide in ventral view (Figure 3D,E). Each lappet armed with a short spinous element at the inner distal corner (Figure 3D,E).

Caudal rami (Figure 3D,E) diverging from posterior margin of anal somite, 1.8 times longer than wide. Each ramus armed with four setae: one outer lateral, two dorsal apical, one inner ventral. All caudal setae biplumose, subequal in length.

Incorporated first pediger and three succeeding free pedigers each with pair of well-developed swimming legs (Figure 4A–D). Protopods consisting of a large coxal portion and a relatively small basis. Anterior articulation between coxa and basis poorly defined whereas posterior diagonal articulation clearly expressed. Coxae of each leg pair joined by longitudinally elongate, rectangular intercoxal sclerite 1.9 times longer than wide. Basis of legs 1, 2 and 4 with short seta proximally on outer margin, reaching at most to middle of first exopodal segment; on leg 3 this seta coarsely plumose and distinctly longer, extending well beyond the tip of exopod. Basis with tri-articulate endopod and exopod on distal margin, with endopod positioned more anteriorly than exopod and reaching only to the distal end of the second exopodal segment. First and third exopodal segments 2 times longer than the second segment, but all three endopodal segments subequal in length. Setal patterns of legs 1–4 are shown in Table 1.

Table 1. Setal armature patterns of legs 1–4.

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-0	I-1; 0-1; I, 2, 2	0-1; 0-1; 1, 2, 2
Legs 2–4	0-0	1-0	I-1; 0-1; I, 2, 3	0-1; 0-1; 1, 2, 2

Roman numerals indicate the number of spines, Arabic numerals indicate number of setae.

Outermost setae of third exopodal segments of all legs smooth, with no serration along outer side. Natatory setae subequally long except short inner setae on first exopodal segment not extending beyond tip if endopod. Fifth legs absent.

3.6. Females

Unrecognized.

3.7. Remarks

The present males are assignable to *Monstrillopsis* by virtue of the presence of type-2 male antennular modification with the fifth segment modified into a gradually tapering, elongate distal half with a slightly inner curved spine on the tip and a hyaline expansion on the inner proximal margin [12,21]. This kind of modification is shared with seven congeners, including *M. sarsi* Isaac, 1974, *M. dubioides* Suárez-Morales, 2004 [4] (for male), *M. chilensis* Suárez-Morales, Bello-Smith & Palma, 2006 [33] (for male), *M. boonwurrungorum* Suárez-Morales & McKinnon, 2014 [34] (correct original spelling), *M. hastata* Suárez-Morales & McKinnon, 2014, *M. nanus* Suárez-Morales & McKinnon, 2014, and *M. pontoeuxinensis* Suárez-Morales & Üstün, 2018. Males of six other congeners, *M. reticulata* (Davis, 1949), *M. fosshageni* Suárez-Morales & Dias, 2001, *M. chathamensis* Suárez-Morales & Morales-Ramírez, 2009, *M. cahuitae* Suárez-Morales & Carrillo, 2013, *M. coreensis* Lee, Kim & Chang, 2016 and *M. longilobata* Lee, Kim & Chang 2016 have a much less elongate distal portion of the fifth segment, with a rather long and robust apical spinous element 5₂. Four species of the former group, *M. dubioides*, *M. sarsi*, *M. nanus*, and *M. pontoeuxinensis* can be immediately excluded from further morphological consideration because of significant differences in body size compared to *M. paradoxa* sp. nov., either larger (2.1 mm for *M. dubioides*, 1.2 mm for *M. sarsi*) or smaller (0.5 mm for *M. nanus* and 0.6 mm for *M. pontoeuxinensis*) [4,6,35,36].

Suárez-Morales and McKinnon [6] recognized mainly two types of male genitalia in *Monstrillopsis*: type I with a strong, well-developed median shaft and relatively short, rounded lappets, and type II with a relatively short median shaft and very elongate lappets arising from the posterolateral corners. The latter is characteristic of all three congeners with type-2 male antennules that were not excluded above on account of body size. These have long, slender, cylindrical lappets [6,26,33], however, while the genital lappets of the new species appear lamellar in the ventral view. In addition, the lappets of *M. paradoxa* sp. nov. are armed with a very short but robust inner distal spinous element, something not previously reported in males of any species of *Monstrillopsis*. The most stunning morphological character of the present males is the unusually low number of somites in the urosome. Up to now, both sexes of *Cymbasoma* have been known to have the fewest urosomal somites among the known monstrilloid genera—females with three, including post-genital somite, and males with four, including post-genital and penultimate somites [8,20,21]. Males of the present new species have one fewer somite than males of *Cymbasoma* while matching the number in females of *Cymbasoma*. Despite this disparity compared to other species of *Monstrillopsis*, which have five urosomal somites in general, nevertheless, we have assigned the present males to *Monstrillopsis* in consideration of its possession of several other *Monstrillopsis*-like features: large, prominent eyes, an anteriorly-located oral papilla, and four caudal setae (see Discussion).

3.8. Etymology

The specific epithet is derived from the Latin adjective *paradoxus*, -a, -um (adopted from the Greek *paradoxos*), meaning strange, with a feminine ending to match the gender of the genus name. It pertains to the contradictory morphological characteristics, a type-2 antennular modification supporting placement in *Monstrillopsis*, and an unusually low number of body somites, which argues against such placement.

4. Discussion

The number of body somites has been considered one of the most important, convenient, and evident key features in the monstrilloid taxonomy [4,7,8,37]. The species of *Cymbasoma*, for instance, have been distinguished from the other monstrilloid genera based almost on this criterion alone [38–40]. A review of the most recent 20 years of taxonomic works dealing with over 40 species of *Cymbasoma* (more than half of the total species recorded in this genus) shows that authors continue to use this feature as the primary feature for generic assignment [20,22,41–46]. In light of this history, the even

lower number of urosomal somites in the present specimens may provide grounds for proposing a new genus of Monstrilloidea. On the other hand, the new species presents a typical type-2 antennular modification, which may be just as important. Full expression of this feature is shared by more than half of the hitherto known males of *Monstrillopsis*, and including partial expression, it has been regarded as a diagnostic feature for the genus [12,47]. Therefore, antennular morphology unambiguously suggests a close morphological affinity of the present specimens to *Monstrillopsis*, and the presence of other *Monstrillopsis*-like characteristics in them further supports their assignment to this genus.

The cuticular pore pattern described above for *Monstrillopsis paradoxa* sp. nov. can be compared with that reported from female *M. planifrons* Delaforge, Suárez-Morales, Walkusz, Campbell & Mundy, 2017. Unlike any other congeneric species, this species has three pairs of dorsal structures, described as “minute papilla-like processes” [48] (p. 4), flanking the midline of the first free pediger. The present new species has six pores arrayed the same way on this somite. Perhaps the structures in *M. planifrons* were also sort of pores. Be this as it may, it is worth noting that Grygier and Ohtsuka [2] have already considered the potential utility of cuticular ornamentation patterns for differentiation and identification. Based on a limited number of examples, they found a stereotypical pore pattern among monstrilloids that distinguishes them from other copepod groups. In addition, based on detailed SEM photographs of four species of *Maemonstrilla*, Grygier and Ohtsuka showed that these species share a similar pore arrangement on the second free pediger [2] (fig. 29). The species of *Caromiobenella* provide another example in that they share a set of four pairs of pores flanking the midline along the posterior medio-dorsal part of the cephalothorax [3,19,23,24]. The currently available evidence thus suggests that pore pattern may be useful in identifying monstrilloids at the genus-level; if so, the present new species still falls into *Monstrillopsis*.

A distinctive pore pattern shared between individuals of different sexes can occasionally reveal their conspecificity. The presence of a similar pore pattern in certain males and females was taken as evidence that both belonged to *Monstrillopsis longilobata*, and this was corroborated by molecular analysis [32]. With this example in mind, the possibility that *M. planifrons* and *M. paradoxa* sp. nov. are the females and males, respectively, of one species must be considered. We think it unlikely, on account of substantial differences in other morphological details besides the dorsal ornamentation of the first free pediger. In particular, unlike males of *M. paradoxa* sp. nov., females of *M. planifrons* are characterized in having (1) a produced, flat, corrugated forehead and (2) a cephalothorax entirely covered in numerous minute papillae, that are considered as species-specific features [48]. None of those features is presented in the present male specimens: the present males have a rounded, convex forehead with transverse striae. The whole cephalothorax is smooth without additional ornamental structures. The outermost seta of the third exopodal segment of legs 1–4 is also different in each species. This seta is, in general, with an inner row of setules and an outer row of coarse spinules as so in *M. planifrons*. The seta of all legs in the present new species is, however, presented without the outer serration. The significant size difference in body length (1.92 mm for the female holotype of *M. planifrons* versus a range of 0.66–0.88 mm for the present males) and considerations of biogeography and habitat—*M. planifrons* being from the Canadian Arctic and *M. paradoxa* sp. nov. from warm-temperate or subtropical waters in East Asia—mitigate against conspecificity [48]. On the other hand, the characteristics mentioned above of the present males may be useful as morphological markers for finding the currently unknown female counterparts. The distinct pigmentation pattern exhibited on the distal part of the antennules in the present males might also be expected to appear in the unknown females.

Female monstrilloids usually have one fewer urosomal somite than the corresponding males [4,8,12,21]. In this rule holds for *M. paradoxa* sp. nov., the unknown females should have a urosome consisting of just two somites: either the first urosomal somite (= fourth free pediger) and the genital (compound) somite, or the genital (compound) somite and anal somite. Loss of the genital somite with retention of the other two would not be supportable, but from a functional point of view, none of these options seems likely. Therefore, we presume that the females of *M. paradoxa* sp. nov. will prove to have at least three somites in their urosome. If so, they will be similar to females of *Cymbasoma*

in terms of the body plan, but distinguishable by the number of caudal setae. Most female *Cymbasoma* have three caudal setae [20,21], and the number of caudal setae is the same (four) in both sexes of those species of *Monstrillopsis* [47].

Our discovery of a new body plan in specimens of the greatly modified, but nonetheless highly stereotyped group Monstrilloida is a sign that further collection and detailed examination of these copepods will continue to turn up surprises. The difficulty in classifying the new species also shows that a phylogenetic systematic approach to monstrilloid taxonomy is overdue. We hope to make progress on this in upcoming works.

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